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Abstract

We report on the diagnostic sensitivity of 3 EU-approved rapid tests (1 from IDEXX and 2 from BIO-RAD) for the detection of transmissible spongiform encephalopathy (TSE) diseases in goats. Ninety-eight goat brain stem samples were tested. All of rapid tests had 100% specificity and ≥80% sensitivity with the IDEXX test significantly more sensitive than the 2 Bio-Rad tests. All tests detected 100% of samples from goats with clinical scrapie, but missed between 7% (IDEXX) and 24% (BIORAD-SG) of samples from pre-clinical goats. Importantly, only IDEXX picked up all samples from clinical BSE-infected goats, whereas the other 2 rapid tests missed between 15% (BIORAD-SG) and 25% (BIORAD-SAP). These results show that a fraction of pre-clinical scrapie infections are likely missed by the EU surveillance, with sensitivity of detection strongly depending on the choice of the rapid test. Moreover, a significant proportion of clinical BSE infections are underestimated by using either BIO-RAD test. Assuming that the same sensitivity on pre-clinical goats would also occur in BSE-infected goats, our data suggest that IDEXX is likely the most sensitive test for detecting preclinical field cases of BSE infection in goats, though with a 7% failure rate. These results raise some concerns about the reliability of current EU surveillance figures on BSE infection in goats.

Key words: BSE; diagnosis; goats; rapid tests; scrapie; sensitivity; surveillance.

Prion infection induces progressive and untreatable neurodegenerative diseases in humans, wild and farmed ruminants, and occasionally in other mammalian species. Prion or transmissible spongiform encephalopathy (TSE) diseases are characterized by the formation and accumulation of an abnormal isoform of the natural prion protein (PrP^{c}) in the central nervous system (CNS) and, occasionally, in peripheral tissues. The pathological prion protein (PrP^{Sc}) differs from PrP^{c} because it appears refolded, aggregated and partially protease resistant. These unique features of PrP^{Sc} have been used for the development of most diagnostic methods currently used for the detection of TSE diseases.

Scrapie disease of sheep and goats has been endemic in Europe for ≥200 years, but has never been convincingly associated with any form of human TSE disease, although recent data based on experimental transmission of scrapie to humanized mice[4] or non-human primates[7] have re-opened this issue. On the other hand, the epidemic of bovine spongiform encephalopathy (BSE) in the UK and other European cattle populations has been unequivocally linked to the appearance of variant Creutzfeldt-Jakob disease in humans[2,23,5]. Because BSE is experimentally transmissible to sheep and goats[10] and these small ruminants were likely exposed to BSE-contaminated feed in the early 1980s, there is concern that the BSE agent may circulate in the sheep and goat population representing a possible secondary risk to humans[8,11].

In 2006 the Commission Regulation (EC) 253/2006[6] approved 9 rapid postmortem tests to monitor the prevalence of scrapie and BSE in small ruminant populations. Sensitivity, based on the lowest detectable concentration of PrP^{Sc} above background noise, and specificity were assessed in classical scrapie cases. In addition, the performance of these tests for the identification of atypical scrapie and BSE in sheep was also evaluated[20,21,18,17,19]. In the frame of such evaluations, only IDEXX[a], BIORAD-SAP[b] and BIORAD-SG[c] tests were able to detect atypical scrapie, a result also confirmed by routine screening for scrapie in sheep and

goats[3, 22]. In 2012, EFSA also recommended PrioSTRIP SR[d] test (visual reading protocol) for the detection of TSE disease in small ruminants. However, a specific study on the suitability of rapid methods for the detection of TSE diseases in goats was never performed.

The goat population in Europe is considerably smaller than that of sheep one, but these ruminants were likely highly exposed to the BSE agent because of feeding of concentrate for dairy farming purposes. Thus, evaluation of surveillance system in place for the goat population is crucial.

We compared the performance of 3 EU-approved rapid postmortem tests for active surveillance of TSE diseases on brain samples from goats with 'natural' scrapie or goats with experimental scrapie or BSE. These three rapid tests resulted 100% specific and sensitive for detecting TSE diseases in sheep.

Ninety-eight goat brain stem samples were included in the study. All samples were prepared as 50% tissue macerates in water as below. Thirty-one of these samples were sourced from goats with 'natural' scrapie from seven different EU countries (Table 1), 7 from clinically affected goats and 24 from clinically healthy animals. Other samples (n=32) from goats with experimentally induced scrapie or BSE were provided by the CVI, FLI, Roslin, INRA and CEA (full names in Table 1). All samples from TSE positive animals resulted also PrP^{Sc} positive at western blot or immunohistochemical analyses as required by the EU Regulation (EC) N. 999/2001[9]. PRNP analyses revealed that 60% of goats carried the wild genotype, while in a few animals polymorphisms I142M (11%), H143R (9%), R154H (2%), R211Q (23%) or repeats deletion (4%) were found in a few animals. Negative control samples were from clinically healthy goats slaughtered in Italy and they were, as expected, negative by Western blot analysis[14]. The whole brain stem sample tissue was trimmed, pooled, mildly minced with a scalpel blade, until the tissue appeared homogeneous. Sterile nuclease-free water was added in an equal amount (50% water/volume) to create a 1:1 dilution. The

suspension was subjected to cycles of homogenization using a low-speed hand-held homogenizing unit until achievement of a homogeneous paste. The resulting homogenate was immediately stored at -80°C and kept frozen until tested. Samples were tested by the IDEXX, the BIORAD-SAP, and the BIORAD-SG ELISAs tests according to the manufacturer's test instructions. The PrioSTRIP SR test was not included in this analysis. The 3 tests are based on semi-quantitative ELISA methods that produce a qualitative result relative to a cut-off value. The two BIORAD tests include a PK digestion step to unmask cryptic epitopes, whereas the IDEXX test relies on conformational detection technology using a specific proteinase resistant binding dextran polymer[12].

The manufacturers specifically provided a unique batch of each rapid test well before the expiry dates to avoid false results producted by old, though still unexpired batches. Samples were coded and then tested in duplicate except for 3 samples from Greece and 1 from the UK because of insufficiently available material. The 3 rapid tests use semi-quantitative ELISA methods that produce qualitative results based on cut-off values. Samples with optical density lower than the cut-off value on both replicates were considered negative. Samples showing an optical density greater than or equal to the cut-off value at least on one replicate were considered positive. However, because the *Bio-Rad* specifications suggest a cautious interpretation of samples situated just below the cut-off value (cut-off value - 10%), we arbitrarily chose to consider these samples as positive. Environmental conditions that might influence testing, such as temperature and humidity, were strictly controlled and monitored during analytical sessions.

The efficiency of each rapid test was assessed by the receiver operating characteristic (ROC) curve analyses (STATA 11, StataCorp LP). Nonparametric ROC curves analyzed TSE-infected goats vs healthy and unaffected goats. The area under the ROC curve (AUC) and its 95% confidence interval (95% CI) indicate diagnostic efficiency.

Overall, the 3 EU-approved rapid tests analyzed showed 100% specificity and >80% sensitivity (Table 2). However, ROC curves showed that the IDEXX test was significantly more sensitive (97%) than the 2 BIORAD rapid tests (Table 3, 4; Figure 1A), which showed sensitivity just >80%.

A more detailed analysis showed that all three rapid tests recognized 100% of samples from goats with experimental scrapie regardless of the route of infection, but only IDEXX showed 100% sensitivity in detecting BSE-infected goats (Table 2, 4). The other 2 rapid tests missed 3 (BIORAD-SG) to 5 (BIORAD-SAP) of the 20 BSE samples (Table 2) with differences that reached significance only between IDEXX and BIORAD-SAP tests (Table 4, Figure 1C).

In goats with natural 'classical' scrapie, the IDEXX test missed 2 of 29 samples and none of the 'atypical' scrapie-infected samples; BIORAD-SAP missed 4 samples and BIORAD-SG 7 (a further sample gave an uncertain result, but was considered positive in the ROC curve analyses) (Table 2). It is of note that the only 2 samples from asymptomatic goats, which were not recognized by the IDEXX test, were also not detected by 2 two Bio-Rad tests. ROC curves showed that the sensitivity of the IDEXX was significantly higher only compared to the BIORAD-SG test (Table 4). Other comparisons did not show any significant differences (Table 4).

Finally, we compared the sensitivity of rapid tests in recognizing goats with scrapie in the pre-clinical or clinical phase of disease. While all rapid tests were systematically able to pick up both natural and experimental scrapie samples from symptomatic goats (Table 3), IDEXX missed 2 of 24 samples with 'natural' scrapie in the pre-clinical phase of disease, BIORAD-SAP missed 4 samples, and BIORAD-SG 7 (Table 3). ROC curves analysis showed that IDEXX and BIORAD-SAP were significantly more sensitive than BIORAD-SG (Table 4) in detecting positive samples from pre-clinical animals.

Several important features of our study should be considered for the surveillance of TSE diseases in goats. All tests detected 100% of samples from goats with clinical scrapie, regardless of whether they were experimentally or naturally infected. In contrast, sensitivity was lower in goats with pre-clinical scrapie and rapid tests missed between 7% (IDEXX) and 24% (BIORAD-SG) of these samples. A second important consideration is that only IDEXX detected all samples from clinical BSE-infected goats, whereas the other 2 rapid tests missed between 15% (BIORAD-SG) and 25% (BIORAD-SAP) of samples. These results suggest that a consistent fraction of pre-clinical scrapie infections are likely missed by the EU surveillance, mostly in areas where BIORAD tests are in use, and that BSE infection in goats may also be underreported in areas using the BIORAD rapid tests (Table 2, 4). Assuming that the same sensitivity on pre-clinical goats would also occur in BSE-infected goats, our data show that the IDEXX test may detect eventual preclinical field case of BSE infection in goats, though with a disappointing 7% failure rate. Although the analytical sensitivity of some TSE rapid tests might be reduced by the method used to prepare our samples[16,1], the results raise some concerns in relation to the current figures on BSE infections in goats deriving from EU surveillance.

In goats, the difference in performance of rapid tests between scrapie and BSE infection might depend on the use of proteinase K (PK) digestion, the choice of the primary anti-PrP antibodies, or both. Interestingly, PK digestion is used by both BIORAD tests but not by IDEXX and is likely that antibodies used in each kit recognize different PrP epitopes. This last hypothesis, however, is purely speculative because the details on anti-PrP antibodies are covered by patents and are therefore not publicly unavailable.

The other interesting result, though based solely on 2 samples, is that only IDEXX and BIORAD-SAP were able to fully recognize samples from goats with the atypical Nor98 scrapie infection suggesting that the in place surveillance system in countries using the

BIORAD-SG test would miss a proportion of atypical scrapie infections in the goat population. The small number of samples, however, is too low to allow a firm conclusion. All rapid tests in this study failed to recognize the same 2 samples of 'natural' preclinical scrapie. This finding is somewhat of concern because it might indicate that there is a small subpopulation of 'naturally' scrapie-infected goats (e.g. early pre-clinical animals) that would be missed by all available rapid tests, and thus by the surveillance system. *PRNP* polymorphisms might reduce the sensitivity of the assays in goats carrying specific genotypes by reducing antibody binding epitopes[15,4]. In our samples, however, statistical analysis did not show any association between failure of each test and goat genotypes (data not shown). The reason for this finding remains therefore unknown and might simply depend on low levels of PrP^{Sc}.

Ultimately, none of the three rapid tests picked up any false positives showing a reassuring 100% specificity.

Sources and manufactures

a. *IDEXX HerdChek ® BSE-scrapie* Antigen Test Kit, EIA. IDEXX Laboratories, Westbrook, ME, USA.

b. *Bio-Rad® TeSeE™ SAP* Purification-Detection Test Kit, Bio-Rad Laboratories, Marnes-La-Coquette, France.

c. *Bio-Rad® TeSeE™ Sheep/Goat* Purification-Detection Test Kit, Bio-Rad Laboratories, Marnes-La-Coquette, France.

d. *Prionics®* - *Check PrioSTRIP SR* Prionics AG, Wagistrasse 27A Schlieren-Zürich, CH 8952 Switzerland.

Declaration of conflicting interests

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Tables

Table 1. Details and origin of goat samples used in the study.

Disease	Type	County of origin (Institute°)	n
Natural scrapie	Classical	Cyprus	3
		France	5
		Greece	4
		Italy	9
		Netherlands	3
		Spain	2
		UK	3
	Atypical (Nor98)	Italy	2
	TOTAL		**31**
Experimental scrapie	Classical	Italy (CEA)	5
		France (INRA)	1
		France (INRA)	6
	TOTAL		**12**
Experimental BSE	Classical	France (INRA)	1
		Netherlands (CVI)	6
		France (INRA)	4
		France (INRA)	1
		Netherlands (CVI)	4
		Germany (FLI)	3
		UK (Roslin)	1
	TOTAL		**20**
TOTAL TSE diseases			**63**
Negative controls	Healthy	Italy	**35**

°INRA, Institut national de la recherche agronomique, France; CVI, Central Veterinary Institute, The Netherlands; FLI, Friedrich-Loeffler-Institut, Germany; CEA, Centro di referenza nazionale per lo studio e le ricerche sulle encefalopatie animali e neuropatologie comparate, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Turin, Italy; Roslin, The Roslin Institute, University of Edinburgh, UK.

276 **Table 2**. Number and percentage of positive samples in goats with different forms of TSE

277 diseases by different rapid tests

Disease	Type	Inoculum	n	Positive test, n (%)		
				IDEXX	**BIORAD SG**	**BIORAD SAP**
Natural scrapie	Classical	-	29	27 (93.1)	22° (75.9)	25 (86.2)
	Atypical (Nor98)	-	2	2 (100)	1 (50.0)	2 (100)
Experimental scrapie	Classical	Scrapie	12	12 (100)	12 (100)	12 (100)
Experimental BSE	Classical	Bovine BSE	20	20 (100)	17 (85.0)	15 (75.0)
TOTAL TSE diseased			**63**	**61 (96.8)**	**52° (82.5)**	**54 (85.7)**
Negative controls	Healthy	-	35	0 (0.0)	0 (0.0)	0 (0.0)

278 °One sample gave uncertain result

Table 3. Number and percentage of positive samples by different tests on 'natural scrapie' affected goats

Disease	Type	Clinical signs	n	Positive test, n (%)		
				IDEXX	**BIORAD SG**	**BIORAD SAP**
Natural scrapie	Classical	No	22	20 (90.9)	15° (68.2)	18 (81.8)
	Atypical (Nor98)	No	2	2 (100)	1 (50)	2 (100)
TOTAL			**24**	**22 (91.7)**	**16 (66.6)**	**20 (83.3)**
Natural scrapie	Classical	Yes	7	7 (100)	7 (100)	7 (100)
Negative controls	Healthy	No	35	0	0	0

°One sample gave uncertain result

294 **Table 4.** ROC curve analyses

	Goats with natural and experimental TSEs (n= 63) *vs*. controls (n=35)	**Goats with natural classical scrapie (n=29) *vs.* controls (n=35)**	**Goats with experimental BSE (n=20) *vs.* controls (n=35)**	**Goats with experimental scrapie (n=12) *vs*. controls (n=35)**	**Goats with TSE with no clinical signs (n=24) *vs*. controls (n=35)**
Diagnostic tests	**AUC (95% CI)**	**AUC (95% CI)**	**AUC (95% CI)**	**AUC (95% CI)**	**AUC (95% CI)**
IDEXX	0.9841 (0.96231-1.0000)	0.9655 (0.91859-1.0000)	1.0000 (1.00000-1.00000)	1.0000 (1.00000-1.00000)	0.9583 (0.90186-1.0000)
BIORAD SG	0.9127 (0.86545-0.95995)	0.8793 (0.8006-0.95856)	0.9250 (0.84472-1.00000)	1.0000 (1.00000-1.00000)	0.8333 (0.73701-0.92966)
BIORAD SAP	0.9286 (0.88502-0.97212)	0.9310 (0.86717-0.99490)	0.8750 (0.77765-0.97235)	1.0000 (1.00000-1.00000)	0.9167 (0.84051-0.99282)
	p value	**p value**	**p value**	**p value**	**p value**
IDEXX vs. BIORAD SG	0.0013	0.0157	0.0671	=	0.0056
IDEXX vs. BIORAD SAP	0.0054	0.1498	0.0118	=	0.1482
BIORAD SG vs. BIORAD SAP	0.5291	0.0723	0.4183	=	0.0320

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